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14. ABSTRACT Multiple receptor pathways allow for redundancy in growth pathways that are dysregulated in cancer and lead to resistance to targeted therapies. EphB4 angiogenesis receptor can cooperate with HER2 growth factor signaling, and co-targeting HER2 and EphB4 could lead to significant therapeutic benefits. The goal of the full project is to assess the in vitro and in vivo growth and signaling effects of co-targeting using approved anti-HER2 agents, trastuzumab and lapatinib in combination with an agent that inhibits EphB4 signaling developed by our group, a ligand-blocking soluble albumin-stabilized EphB4 peptide termed sEphB4-HSA. We have determined that Her2 induces EphB4 and EphrinB2 in three different settings including isogenic cell lines with and without Her2 expression, gene expression data sets on human breast cancer tissues from three different cohorts, and in the Her2 trangene expressed in mammary tissue/tumor in mice. We next determined that EphB4 provides survival signal in Her2 positive tumor cell lines in vitro and in ex-vivo human tumor fresh samples. Next we studied in transgenic mouse expressing Her2 in the mammary gland causing spontaneous tumor. Inhibition of EphB4-EphrinB2 with soluble decoy Ephb4 markedly inhibited tumor formation, growth and metastasis. A remarkable novel finding is that blocking EphB4-EphrinB2 interaction with sEphB4 led to marked inhibition of Her2 phosphorylation. Thus EphB4-EphrinB2 so induced by Her2 is required to activate Her2. We next studied genetic model in which EphB4 or EphrinB2 gene is deleted to determine if Her2 induced spontaneous tumor formation is reduced. This indeed is the case. Thus sEphB4 is a candidate for studies in Her2 positive breast ca.

15. SUBJECT TERMS
HER2,EphB4, EphrinB2, Ephrin, Breast Cancer, immunohistochemistry, predictive factor

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INTRODUCTION:

We hypothesize that the EphB4 angiogenesis receptor can cooperate with HER2 growth factor signaling and that co-targeting HER2 and EphB4 could lead to significant therapeutic benefits. The full project has aims to assess the in vitro and in vivo growth and signaling effects of co-targeting using approved anti-HER2 agents, trastuzumab and lapatinib in combination with an agent that inhibits EphB4 signaling developed by our group, a ligand-blocking soluble albumin-stabilized EphB4 peptide termed sEphB4-HSA. The other component cover by this project by the Partnering PI focuses on the human tissue analysis in patients who received pre-operative therapy with or without the HER2-targeted antibody trastuzumab. Markers of endothelial angiogenesis, including EphB4 and its cognate ligand EphrinB2 and downstream signaling will be analyzed for the relationship to response and to determine if enrichment of these markers occurs in non-responders from the initial biopsy to the post-treatment surgical specimen. The demonstration of these human tissue effects along with the efficacy of in vitro and in vivo co-targeting of HER2 and EphB4 will set the stage for clinical trial strategies as sEphB4-HSA is already in Phase I testing at our institution.

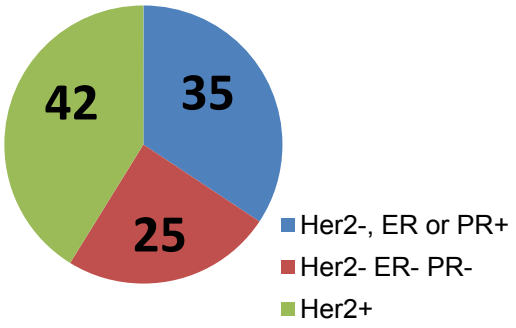
BODY:

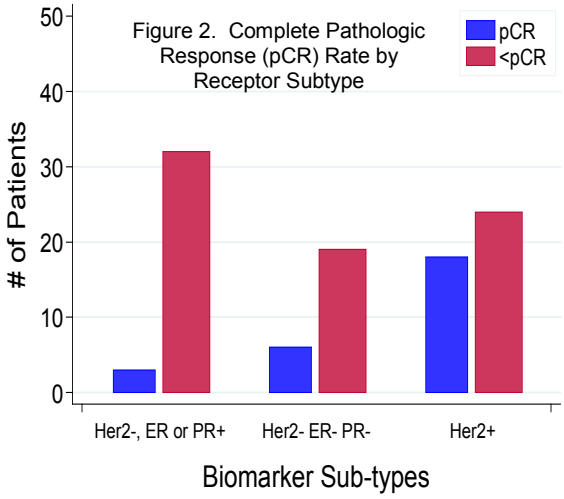
Refer to Statement of Work for Aim 1 (aim related to Partnering PI, Tripathy)

Task 1A. We have obtained human subjects approval from USC IRB and DOD CDMRP and for consent waiver to obtain tissue blocks and clinical information (USC IRB# S10-00511).

Task 1B. We have identified consecutive patients who underwent neoadjuvant therapy and definitive surgery at our institution, a majority of such cases coming from Los Angeles County Hospital (LAC+USC). A total of 102 cases overall of neoadjuvant therapy were obtained, including 42 HER2+ cases. The breakdown of receptor subtypes in this cohort is shown in Fig 1. The ethnic breakdown of the overall cohort was representative of minority population seen at LAC+USC, 80% Hispanic, with the HER2+ cohort being 81% Hispanic. The overall complete pathological response (pCR) rate in the cohort

Figure 1. Receptor Distribution (%) in Neoadjuvant Cohort





was 28.4%. The rates of pCR by receptor subtype are show in Fig. 2. Within the HER2+ cohort pCR rate was 42.9%, a statistically significant difference (pCR odds ratio 15 compared to ER or PR+ and HER2-neg, $p<0.001$ by multivariate analysis). Of the 42 HER2+ cases, 32 patients received neoadjuvant trastuzumab along with chemotherapy and exhibited a higher pCR rate (pCR 50.0 vs. 27.3%).

Task 1C. We have obtained all the blocks available on the 42 HER2+ cases, including baseline core biopsies and surgical specimens from patients who did not exhibit a pCR. A total of 24 patients did not achieve a pCR cases and 18 cases did have a pCR. A total of 63 blocks has been retrieved. For paired pre/post samples in non-pCR cases (needed for Task 3E), of the 24 non-pCR cases, 14 paired breast samples are available. The reason for this low yield in this particular group is not clear – in some cases, only nodal tissue (7 cases) was available for one of the time points in the pathology archives and in 3 cases, only pre (2 cases) or post therapy (1 case) tissue was available, potentially due to cases that were exhausted due to clinical tests needed for patient care or due to transfer of care to another facility. We have addressed this by amending our IRB protocol to allow us to extend the end date for our consent waiver to obtain additional cases. So far we have identified 30 additional HER2+ cases although given the regulatory delays in obtaining these, the analysis will not be performed in the context of this project, but will be pursued in the future with the additional of markers and increase in statistical power.

Task 2A. Staining for H&E for the presence of tumor cells has been done on all 63 blocks retrieved. These were scored for intensity and % positive among malignant cells by co-Investigator pathologist Debra Hawes, MD. Some of the blocks had no tumor tissue or very scant tumor as these were from core biopsies, so a not all stains were performed. Some had in situ tumor only for analysis and those were read in the same fashion as invasive and scored malignant non-invasive cells (See Table 1).

Table 1. Successful Staining for Antigens Tested

EphB4	EphB2	c-MET	IGF-1R	PDGFR	VEGFR1	CXCR4	Ki67
56/63	56/63	57/63	60/63	58/63	58/63	56/63	59/63

Eph B4: 6 with insufficient tumor, 1 skin and 2 in situ only; Eph B2: 6 with insufficient tumor, 1 skin and 2 in situ only; c-MET: 6 with insufficient tumor and 1 in situ only; IGF-1R: 3 with insufficient tumor and 2 in situ only; PDGFR: 4 with insufficient tumor, 1 skin; VEGFR1: 4 with insufficient tumor, 1 skin and 1 in situ only; CXCR4: 4 with insufficient tumor, 1 skin; Ki67: 4 with insufficient tumor and 1 in situ only.

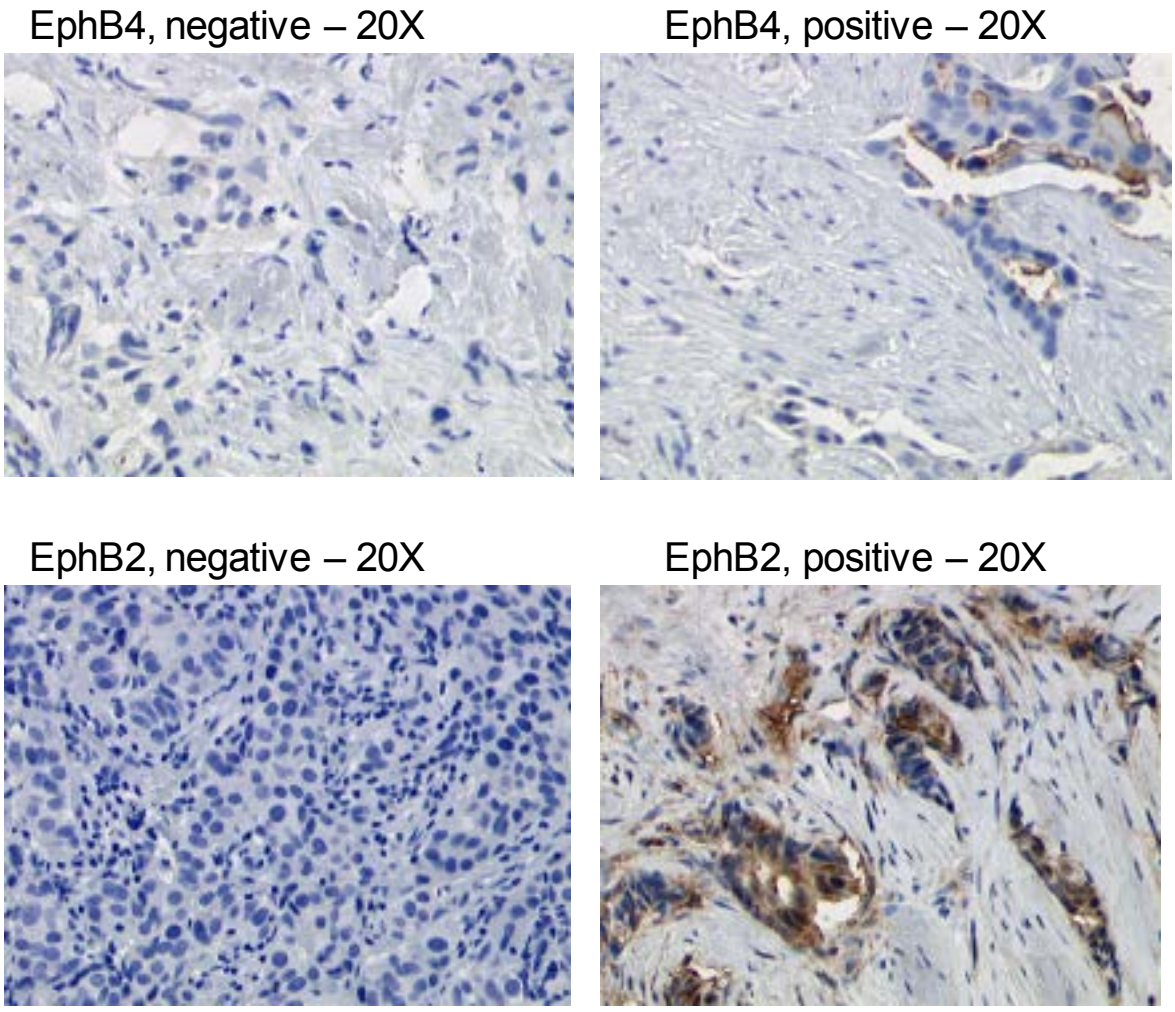
Task 2B. All cases have been reviewed for pathological response using record and pathology reports.

Task 2C. All antibodies were obtained and optimized for formalin-fixed paraffin embedded tissue analysis and scoring.

Task 2D. The planned immunohistochemical (IHC) stains - EphB4, EphB2, HGFR/c-MET, IGF-1R, PDGFR, VEGFR1 and additional stains for CXCR4 (a potential resistance markers identified in our lab) as well as Ki67 proliferation index were all optimized and then sections stained. These were scored for intensity and % positive among malignant cells by co-Investigator pathologist Debra Hawes, MD. Some of the blocks had scant tissue, so a not all stains were performed.

Representative stains for EphB4 and EphB4 are shown on Fig 3. In some cases, alternate vendors not listed in the proposal were used to obtain antibodies that work well in formalin-fixed, paraffin-embedded tissue.

Figure 3. Representative IHC Stains for EphB4 and EphB2 on Pretreatment Cases



Task 2E. Statistical Analysis. Our primary objective for this aim was to identify baseline staining for the antigens of interest and their relationships to trastuzumab (Herceptin) response as measures by achievement (vs. not) of a complete pathological response (pCR), defined in the conventional fashion as the absence of invasive cancer in breast nodal tissue in the surgical specimen following trastuzumab-containing treatment and surgery. Our secondary objective was to compare markers pre and post trastuzumab-based therapy in patients who did not achieve pCR in order to assess if there was enrichment of markers suggesting that they might drive resistance. Additional objectives as to assess the same variables (pre treatment biomarkers and pCR, and changes in pre and post specimens in patients who did not achieve pCR) for all patients regardless of therapy, as some patients, particularly in earlier years, did not receive trastuzumab-based therapy and only received chemotherapy.

Statistical Methods

For the participating subjects, tissues were obtained prior to treatment and post treatment (if a subject did not have a pCR). Immunohistochemically stained slides were graded for intensity staining (0, negative; 1, weak; 2, moderate; 3, strong) and percentage of cells stained (0%-100%). Histoscores were calculated as the multiplication of marker intensity score and percent of cells stained. Data on 8 markers were analyzed, including EphB2, EphB4, CXCR4, IGF1, c-MET, PDGFR, VGEFR1, and Ki67. Changes in marker histoscores post treatment vs. pre treatment were examined with paired t-test. In addition, Wilcoxon rank sum test was used to compare baseline marker histoscores between subjects who achieved a pCR vs. those who did not achieve a pCR.

Results:

Part I: Subjects who received pre-operative trastuzumab plus chemotherapy

Of the 42 subjects in the total cohort, 32 patients received trastuzumab with chemotherapy and the remainder received chemotherapy alone. Of these 32 patients, 16 had a pCR and 16 did not have a pCR.

Of the 16 patients who did not have a pCR, 12 had tissue available with evaluations of biomarkers both pre-treatment and post-treatment. For some cases, percent of staining or intensity is missing for some biomarkers). Among the 8 biomarkers examined, no significant difference was found in marker histoscores at baseline for subjects who achieved pCR vs. those who did not (Table 2). In comparing pre and post treatment values among patients not achieving a pCR, there was a significance difference in biomarker histoscores post treatment compared to pre treatment for VEGFR1 ($p=0.030$) and Ki67 ($p<0.001$) (Table 3). Distribution of biomarker histoscores pre and post treatment are shown in Figure 4. Distributions of biomarker histoscores by pCR and non pCR categories are shown in Figure 6.

Table 2. Baseline Biomarker Histoscores in Relation to Response (pCR) in Cases Treated with Trastuzumab plus Chemotherapy

Markers	Histoscore at baseline (Median (min, max))		p-value [1]
	pCR	Non pCR	
EphB2	1 (0, 69)	0.6 (0, 285)	0.78
EphB4	0.2 (0, 45)	0 (0, 9)	0.30
CXCR4	14 (0, 200)	0 (0, 249)	0.41
IGF-1R	180 (57, 291)	178 (42, 258)	0.93
c-MET	24 (0, 300)	73 (0, 200)	0.60
PDGFR	200 (0, 300)	194 (72, 300)	0.98
VGEFR1	80 (18, 300)	84 (9, 300)	0.71
KI67	95 (40, 240)	95 (64, 189)	0.76

[1] p value from Wilcoxon rank sum test.

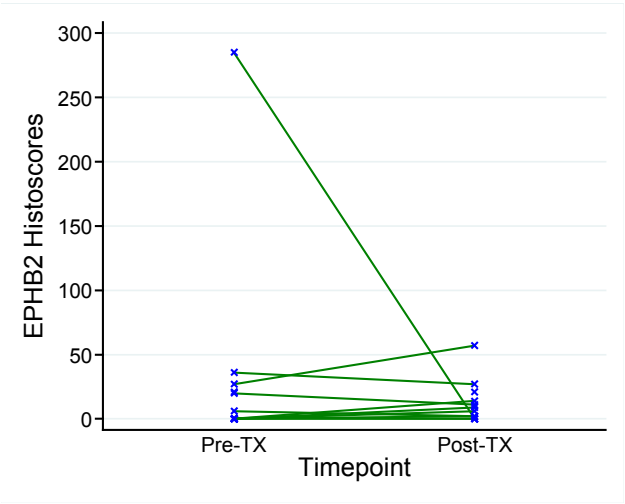
Table 3. Biomarker Histoscore Changes Pre and Post Trastuzumab plus Chemotherapy

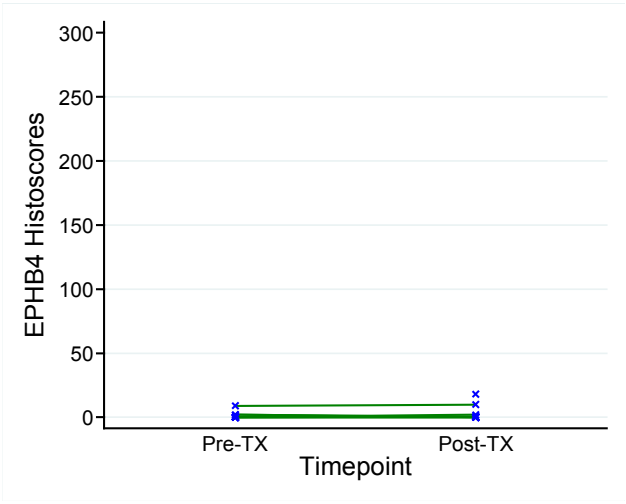
Markers	Histoscore (Mean ± SE)		Difference[2] (Mean ± SE)	p-value [1]
	Pre-Treatment	Post-Treatment		
EphB2	34.1±25.4	11.6±5.2	-22.4±26.5	0.42
EphB4	1.2±0.82	1.2±0.90	0.001±0.34	0.99
CXCR4	50.5±25.0	79.8±34.5	29.3±26.8	0.30
IGF-1R	163±20.6	152±19.7	-10.7±25.5	0.68
c-MET	91.1±25.4	60.5±25.4	-30.6±44.0	0.50
PDGFR	200±27.2	113±28.0	-87.3±52.2	0.13
VGEFR1	143±34.6	56.1±11.9	-86.5±34.3	0.030
Ki67	97.8±7.5	41.0±9.9	-56.8±11.8	<0.001

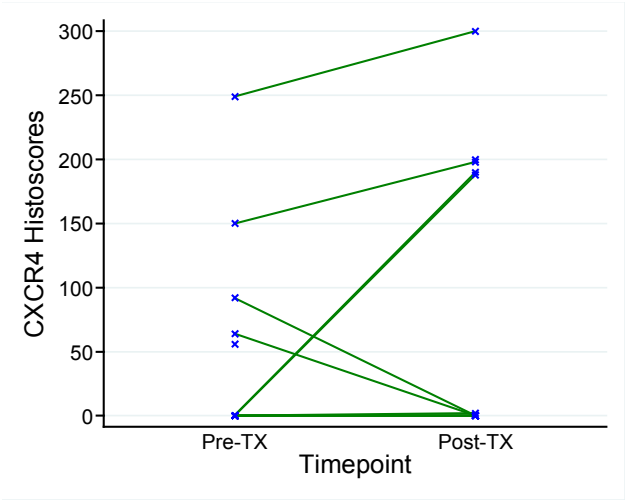
[1] p value from paired t-test.

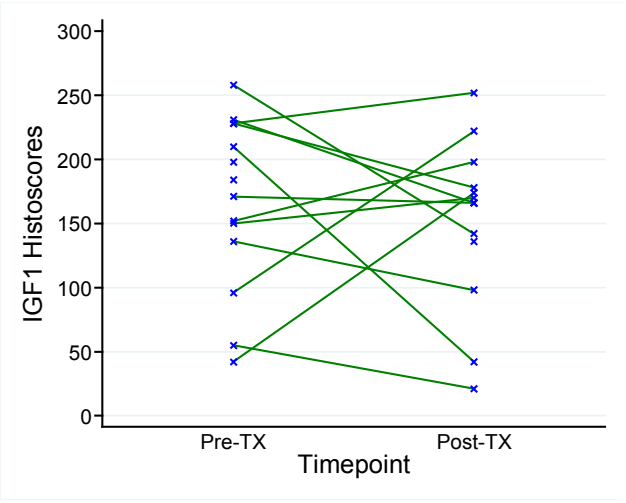
[2] Difference post treatment vs. pre treatment

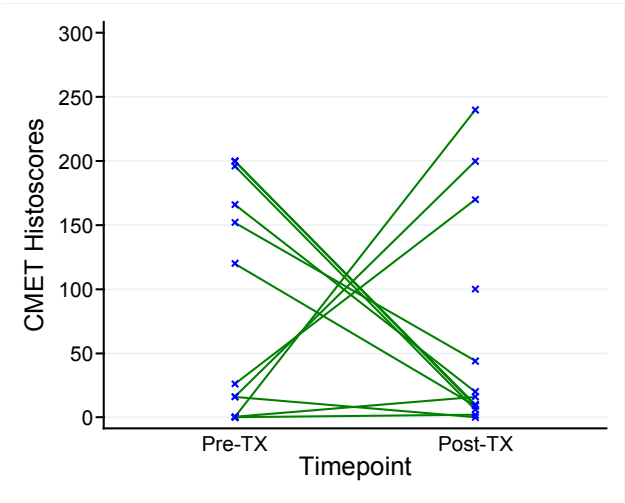
Figure 4. Distribution of Biomarker Histoscores at Baseline and Post Trastuzumab plus Chemotherapy

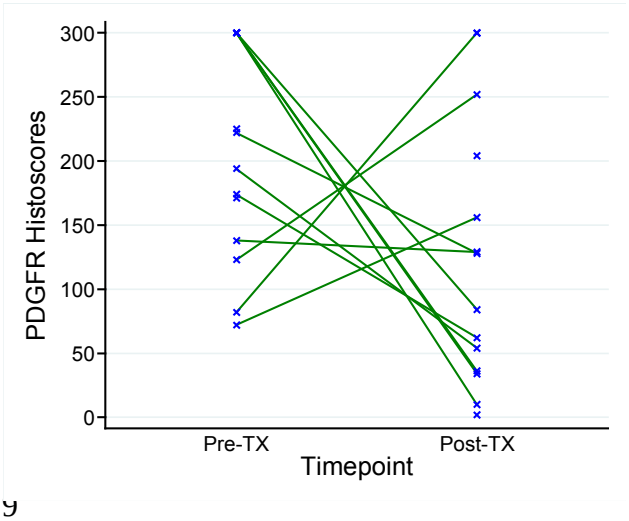












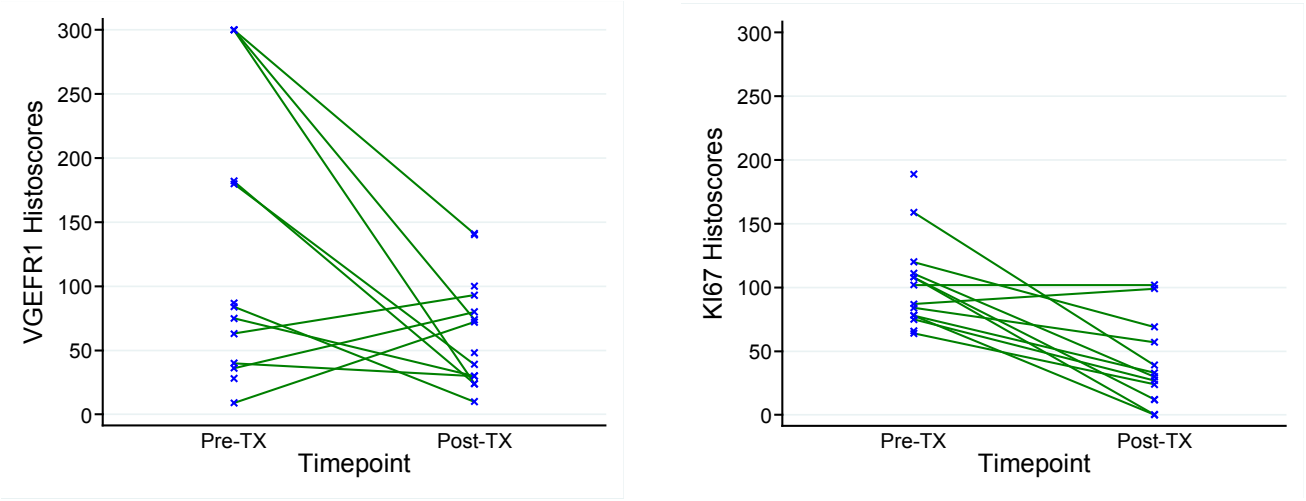
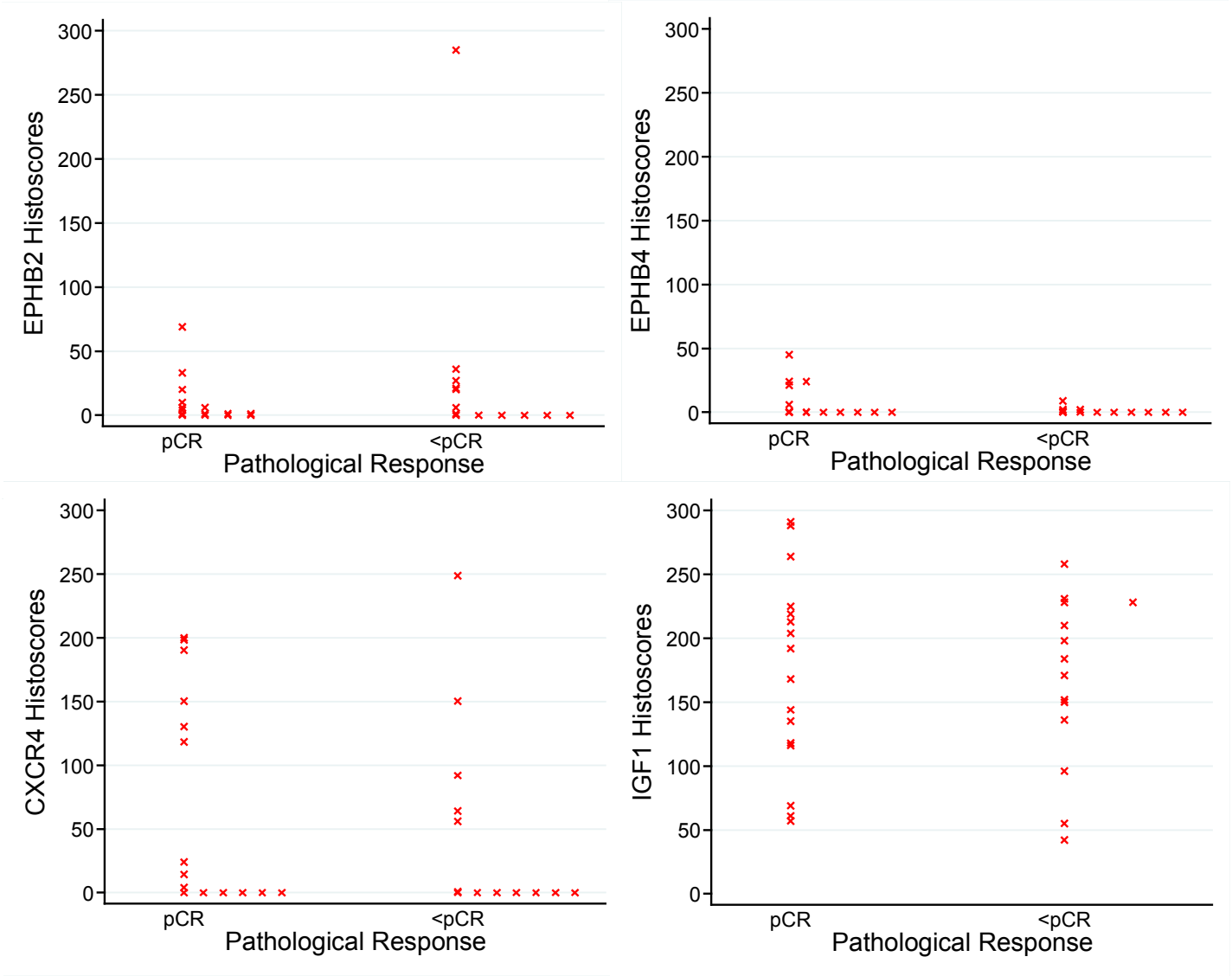
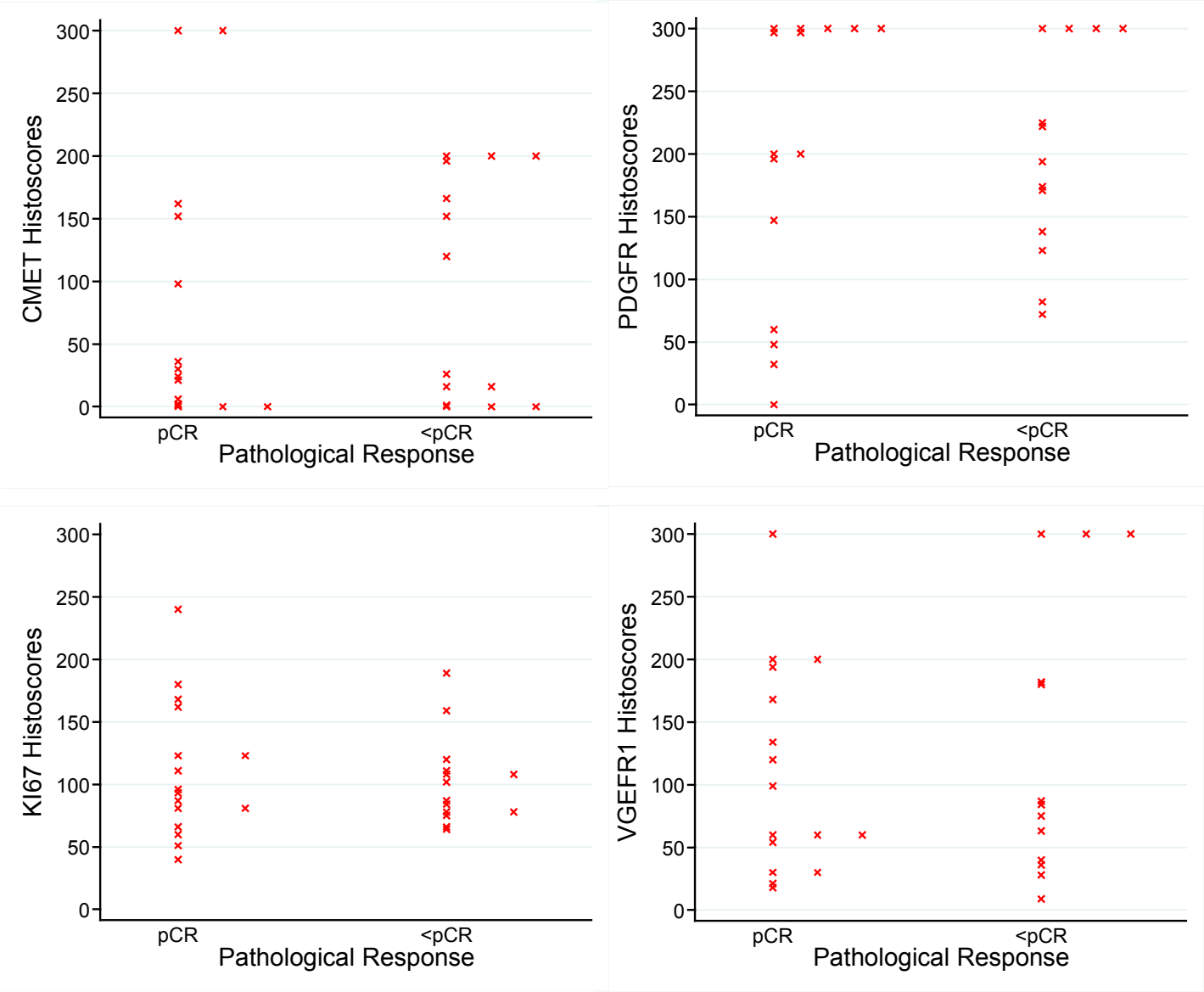


Figure 5. Distribution of Biomarker Histoscores by Response to Trastuzumab plus Chemotherapy





Results Part II: All subjects

Among all 42 subjects in the cohort, all received chemotherapy only, and 32 received trastuzumab plus chemotherapy; 18 had a pCR and 24 did not have a pCR.

Of the 24 subjects who did not have a pCR, 18 had tissue for evaluation of the biomarkers both pre-treatment and post-treatment. For some cases, percent of staining or intensity is missing for some biomarkers. No significant difference was found among the 8 biomarker histoscores at baseline for subjects who achieved pCR vs. those who did not achieve pCR (Table 4). There was a significant difference in biomarker histoscores post treatment compared to pre treatment for PDGFR (p=0.051), VGEFR1 (p=0.007), and Ki67 (p=0.008) (Table 5). Distribution of biomarker histoscores pre and post treatment are shown in Figure 6. Distributions of biomarker histoscores by pCR and non pCR categories are shown in Figure 7.

Table 4. Baseline Biomarker Histoscores in Relation to Response (pCR) in Cases Treated with Chemotherapy with or without Trastuzumab

Markers	Histoscore at baseline (Median (min, max))		p-value [1]
	pCR	<pCR	
EphB2	0.9 (0, 69)	0.6 (0, 300)	0.83
EphB4	0.2 (0, 45)	0 (0, 9)	0.15
CXCR4	14 (0, 200)	0 (0, 276)	0.29
IGF-1R	156 (57, 291)	164 (42, 258)	0.89
c-MET	21 (0, 300)	111 (0, 200)	0.17
PDGFR	297 (0, 300)	174 (10, 300)	0.24
VEGFR1	80 (18, 300)	69 (9, 300)	0.86
Ki67	90 (40, 240)	86 (33, 189)	0.62

[1] p value from Wilcoxon rank sum test.

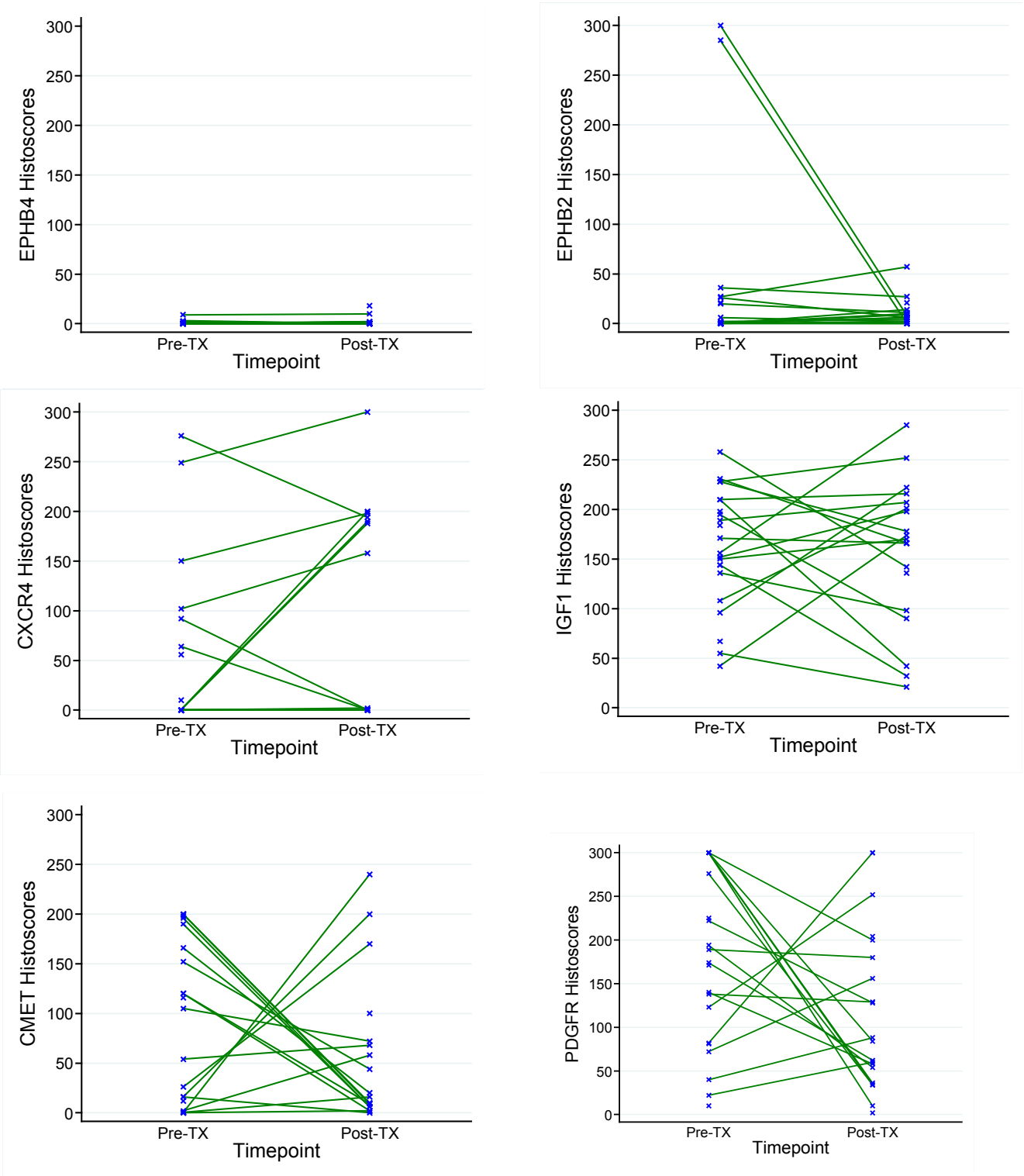
Table 5. Biomarker Histoscore Changes Pre and Post Chemotherapy with or without Trastuzumab

Markers	Histoscore (Mean ± SE)		Difference [2] (Mean ± SE)	p-value [1]
	Pre-TX	Post-TX		
EphB2	41.4±23.1	9.5±3.4	-31.8±23.6	0.20
EphB4	0.99±0.55	0.92±0.59	-0.07±0.31	0.82
CXCR4	54.9±22.1	84.1±25.9	29.2±21.4	0.19
IGF-1R	164±14.4	159±17.7	-5.5±21.1	0.80
c-MET	92.0±19.7	54.8±18.3	-37.2±33.0	0.28
PDGFR	187±23.9	110±20.2	-76.9±36.5	0.051
VEGFR1	119±25.1	45.4±8.6	-73.7±23.8	0.007
Ki67	98.2±6.9	60.7±14.6	-37.5±12.4	0.008

[1] p value from paired t-test.

[2] Difference post treatment vs. pre treatment

Figure 6. Distribution of Biomarker Histoscores at Baseline and Post Chemotherapy with or without Trastuzumab



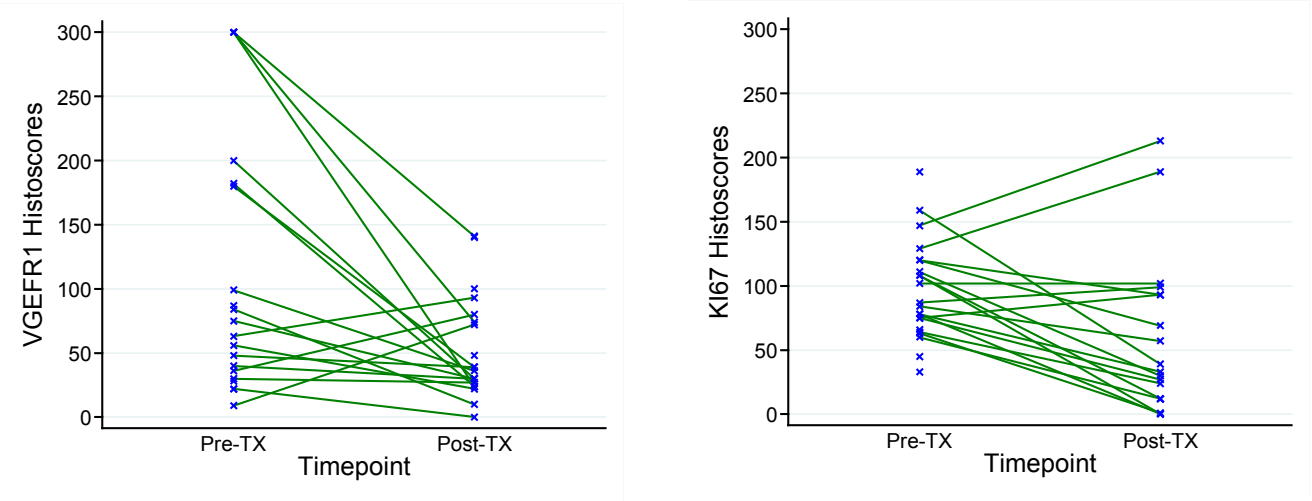
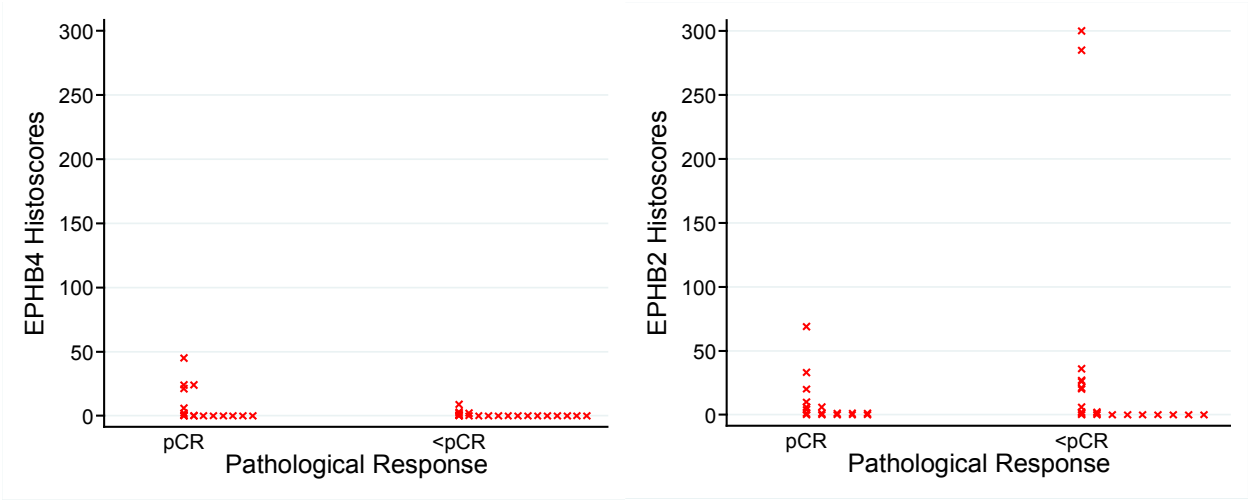
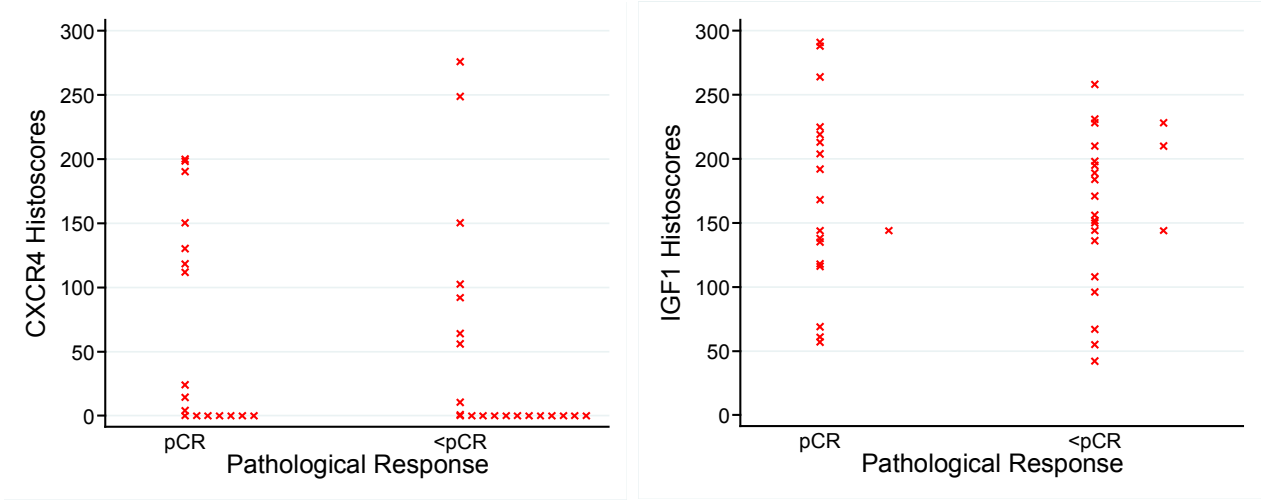
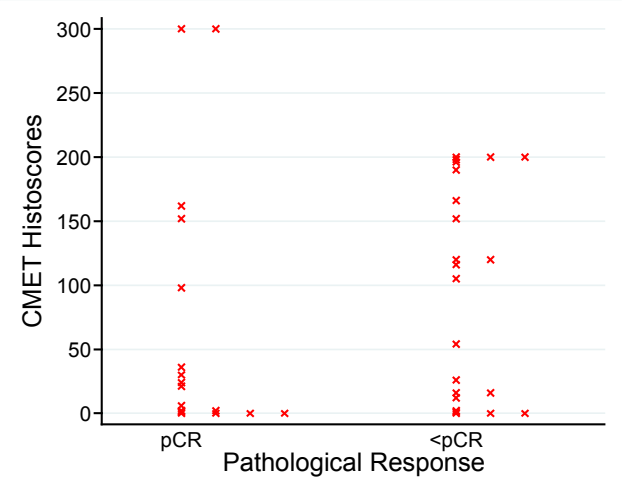
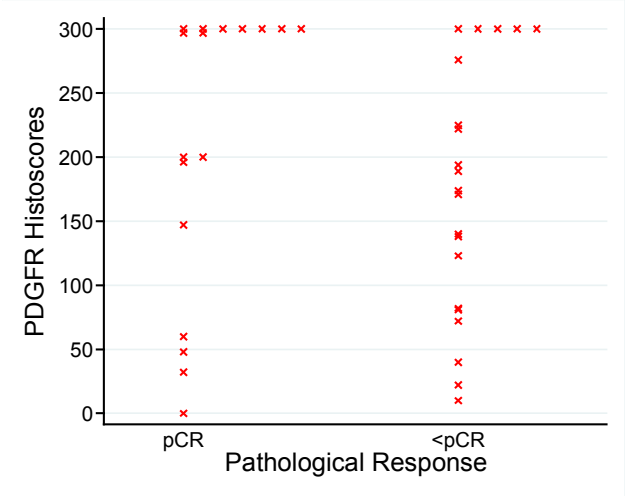


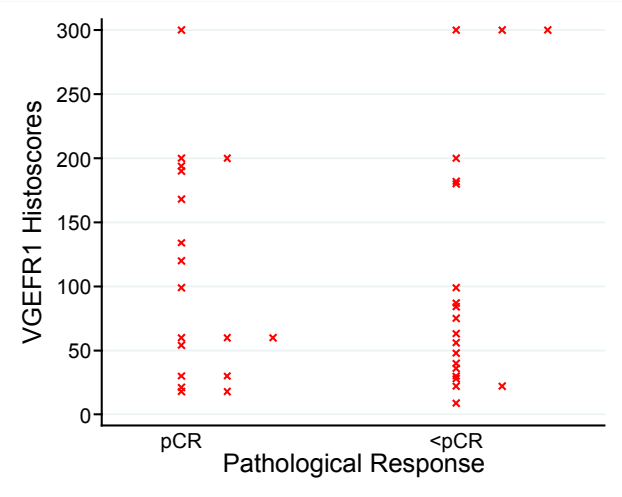
Figure 7. Distribution of Biomarker Histoscores by Response to Chemotherapy with or without Trastuzumab

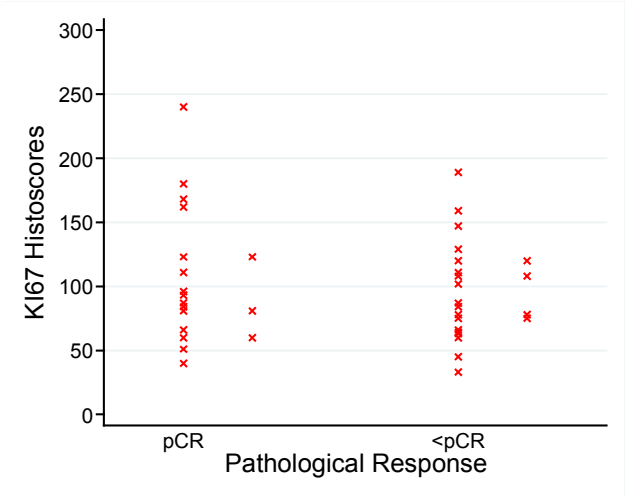






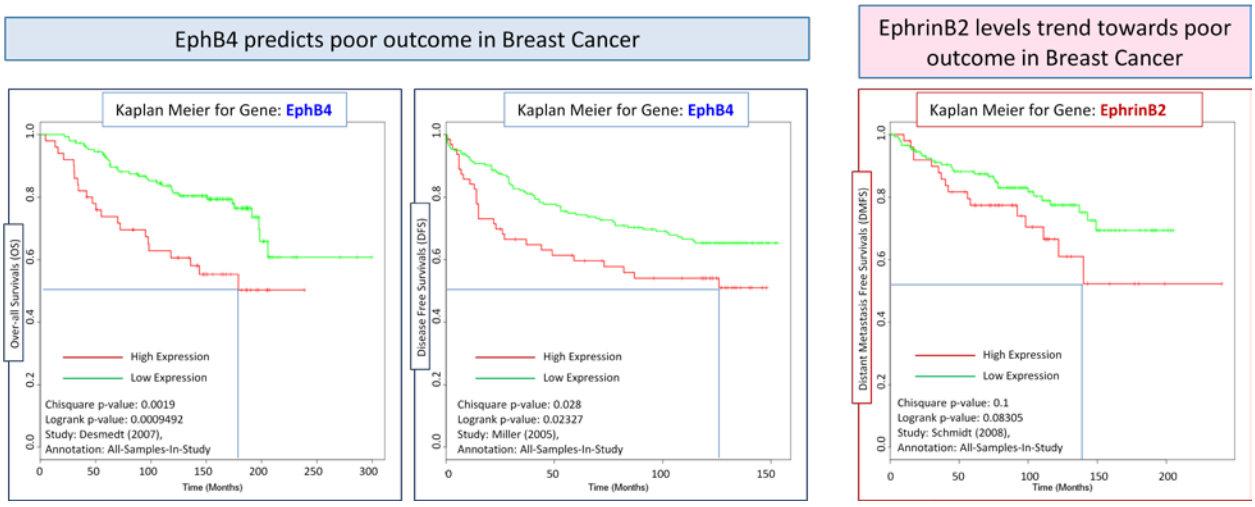


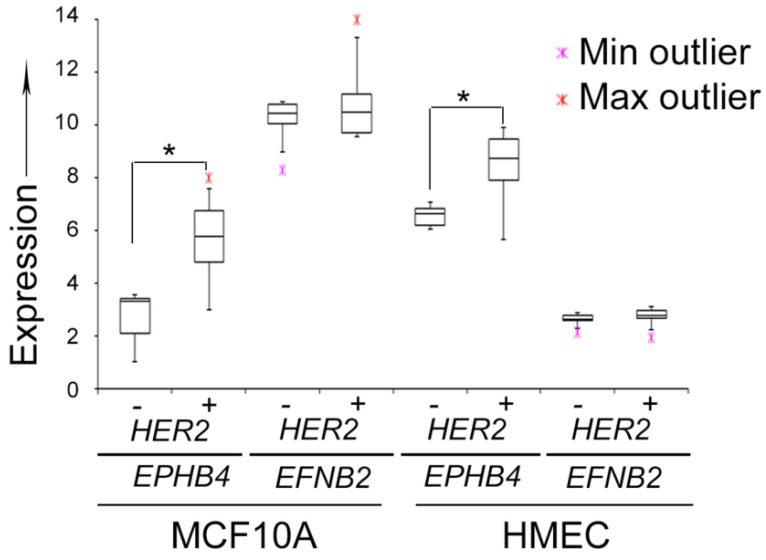




Task 3.

Task 3A. Gene expression analysis of breast cancer data base was analyzed for the expression of EphB4 and EphrinB2 and correlated with survival. Three different data sets were analyzed. In each data set, the expression of EphB4 and EphrinB2 was found to correlate with decreased survival.

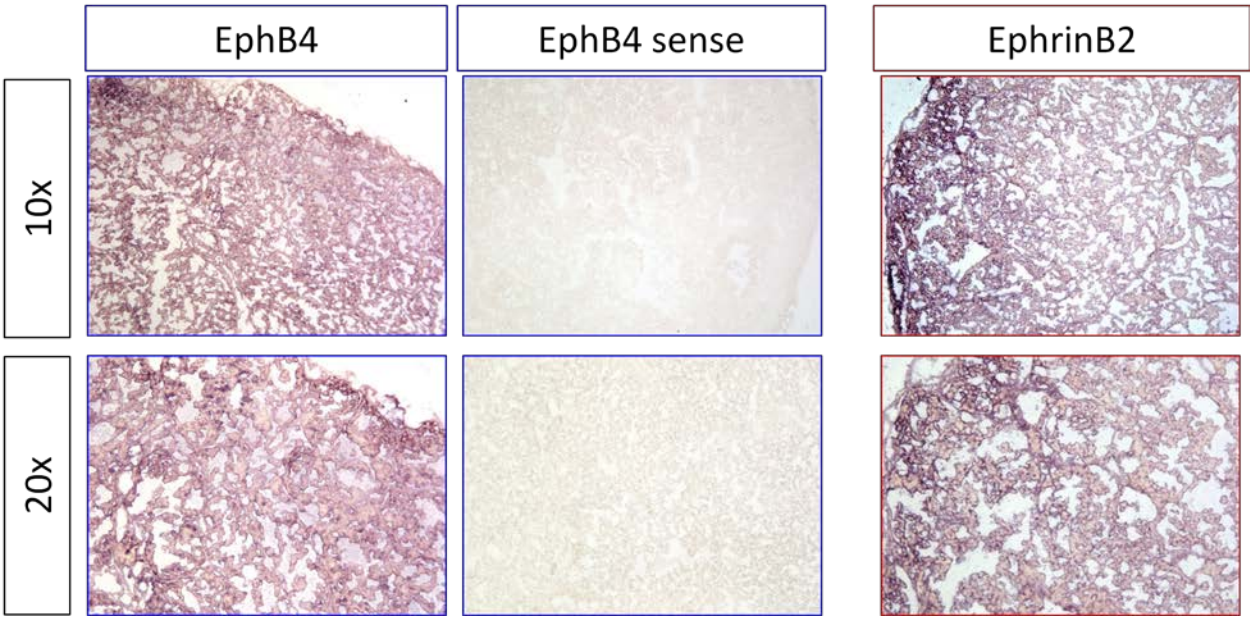




Box Whisker plot with 95% confidence intervals indicating the level of expression of indicated genes in MCF10Al and HMEC breast tissue cell ines. Box whiskers plots indicate median expression of gene in various cell lines (line in the box). The whiskers present an entire range of expression levels of gene and the ends of the whisker are set at 1.5*IQR (interquartile range) above the third quartile (Q3) and 1.5*IQR below the first quartile (Q1). If the Minimum or Maximum values are outside this range, then they are shown as outliers as detailed previously (Punj et al . 2009; Blood 113(22); 5660-5668) The expression of EPHB4 and ENFB2 in MCF10A and HMEC cells was significantly increased in Her2 knock-in cells while these genes are not statistically up-regulated in either cell lines.

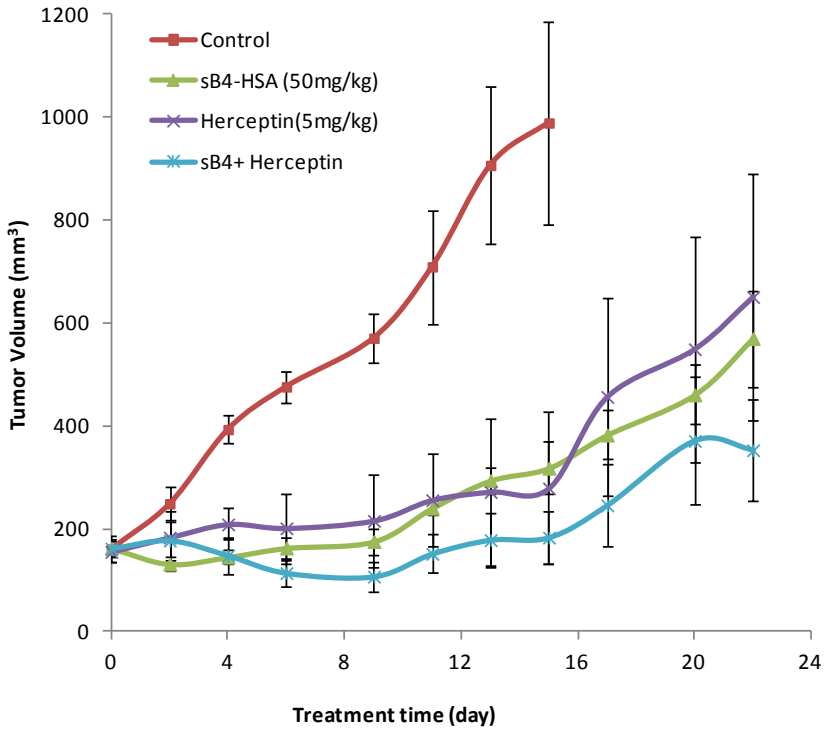
Task 3B. We next determined if Her2 specifically induces the expression of EphB4 and EphrinB2. Isogenic cell lines with stable expression of Her2 or empty vector were studied for eh gene expression with focus on EphB4 and EphrinB2. Her2 expressing cell line had higher expression of EphB4 and EphrinB2 compared to empty vector. Furthermore, the induction of EphB4 and EphrinB2 correlated directly. Thus Her2 induces both EphB4 and EphrinB2.

Task 3C: EphB4 and EphrinB2 expression is induced in Her2 transgene expression in mouse mammary tissue: Trangenic mouse expressing Her 2 under the MMTV promoter induced preferentially in mammary tissue leads to mammary tumor. Tumor samples were harvested and analyzed for the mRNA expression of EphB4 and ephrinB2 using in situ hybridization. Marked induction of EphB4 and EphrinB2 was observed. Control mRNA probe as expected had no background signal. Thus inclusion from the studies in task 3A,B,C show that Her2 induces EphB4 and EphrinB2 in vitro, in murine model and in human tumor tissue analysis.

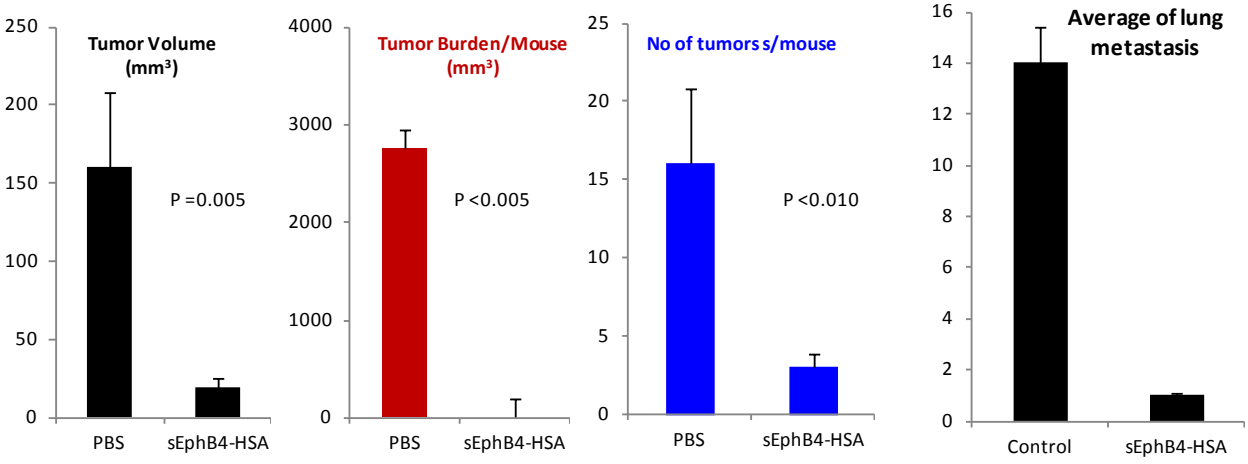


Task3D. Does induction of EphB4 and EphrinB2 regulate Her2 positive breast cancer cell line proliferation and viability. Her2 positive breast cancer cell lines were seeded in vitro in multi-well plates and treated with the soluble form of EphB4 receptor that blocks EphB4-EphrinB2 interaction and thus functions as an antagonist of bidirectional signaling. Cell viability was measured on day 3. A dose dependent decrease in cell viability was observed in Her2 positive breast tumor cell lines indicating that EphB4-EphrinB2 provide survival or proliferative function Data fro Task3 thus far supported the investigation of sEphB4 in vivo

Task 3E: Her2 positive human breast cancer cell line shows tumor growth inhibition in vivo. H1419 tumor cell line was implanted in athymic mouse and allowed to establish. Mice were then randomly assigned to receive either sEphB4, Herceptin of combination of both. Tumor growth was measured twice or three times a week for the duration of the study. sEphB4 and Herceptin both showed tumor growth inhibition and they were comparable to each other. Combination of both further reduced the rate of tumor growth. Thus sEphB4 has a direct anti-tumor effect in Her2 positive tumors.

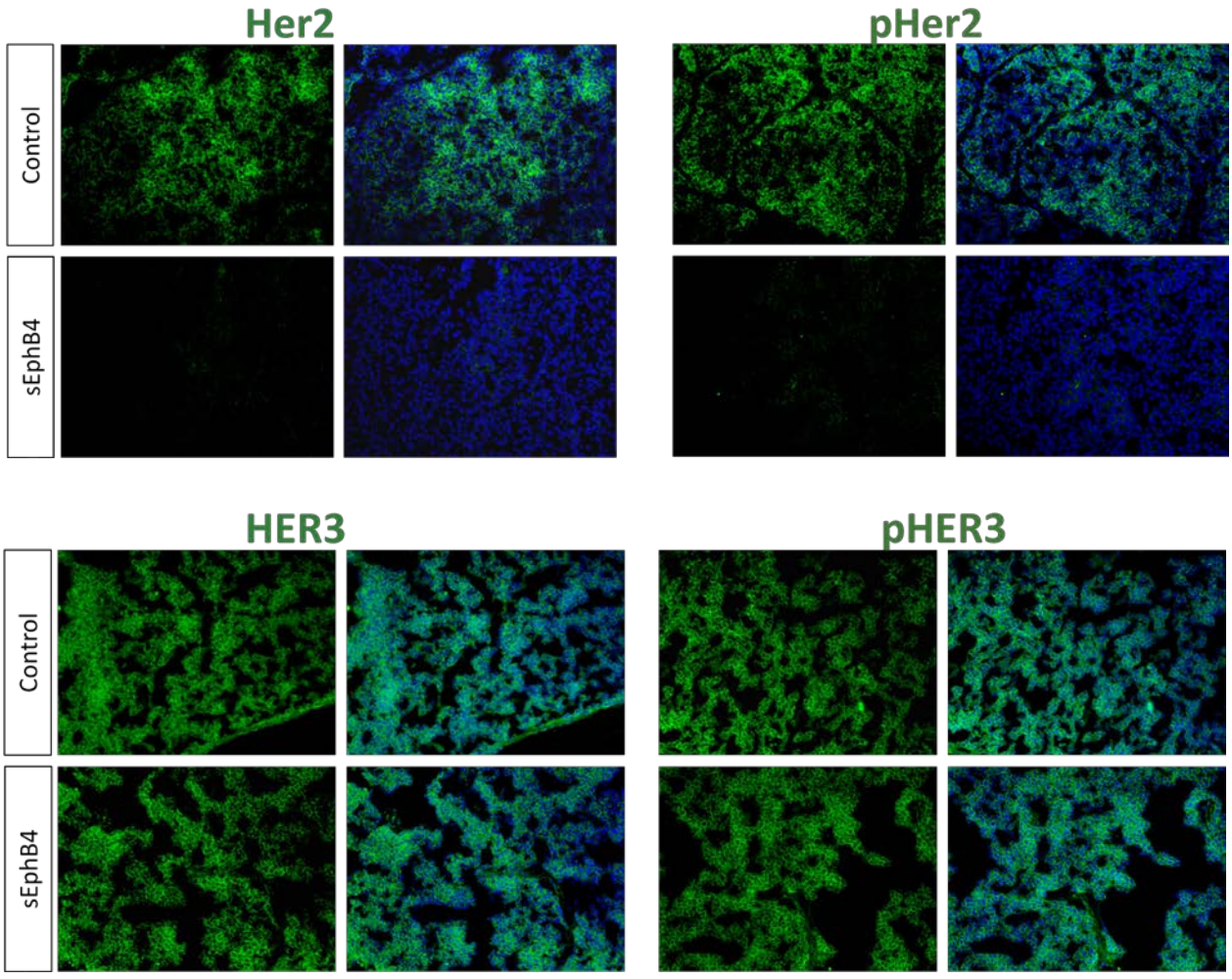


Task 3F: Study EphB4 antagonist in Her2 positive spontaneous tumors in mouse. Her2 transgenic mouse were monitored until the development of mammary tumor. Mice were then treated with either sEphB4 or PBS **Days 140-175**. Tumors were measured over time for the total number o tumors, tumor size, cumulative tumor burden and the tumor metastasis. Tumor number, size, cumulative tumor burden and the tumor metastasis to the lungs were markedly reduced in the sEphB4 treatment group compared to the controls.



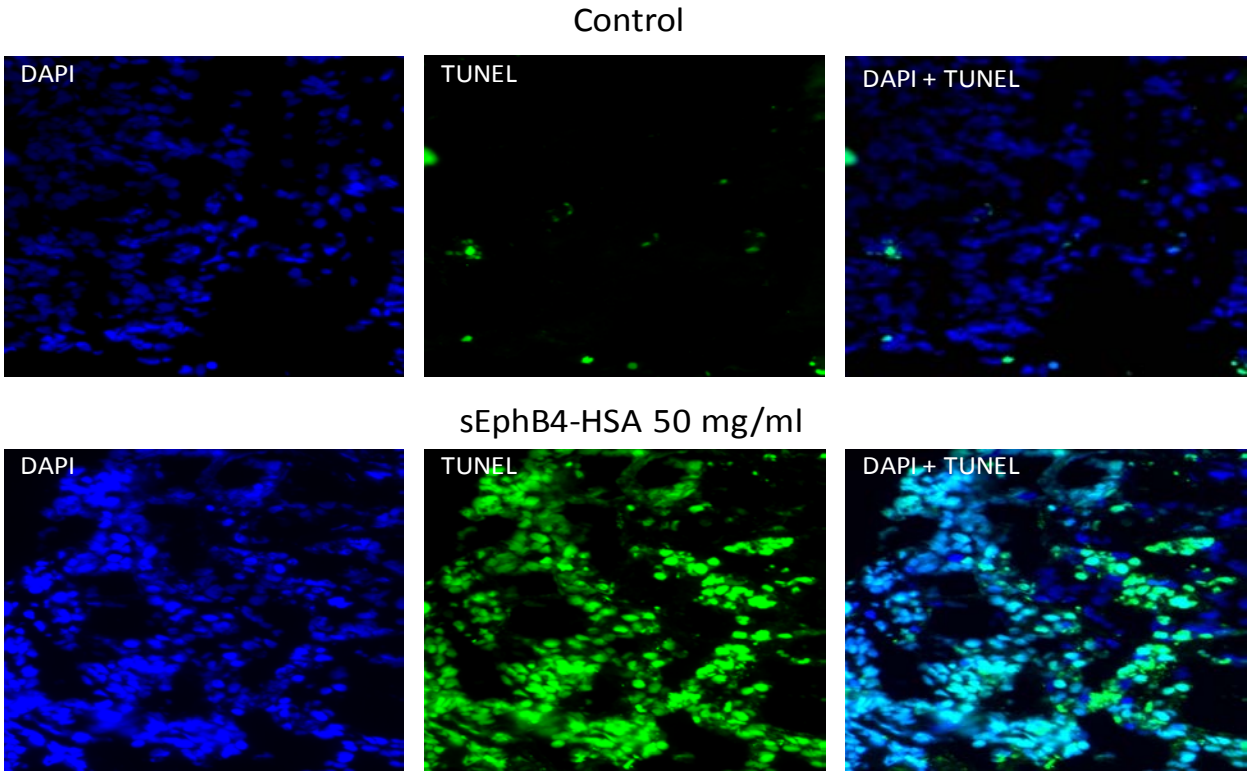
Task 3F: EphB4-EphrinB2 targeting with sEphB4 antagonist blocks the Her2 recptor activation: In order to study the mechanism of action of EphB4 in the efficacy in Her2 induced mammary tumor, we studied the status of Her2, EGFR1 and EGFR3 phophorylation. sEphB4 markedly

inhibited the phosphorylation of Her2 as well as EGFR1 but not EGFR3. Thus the effect is specific and directed to the oncogene Her2. Inaction of the driver oncogene provides the strong rational to apply EphB4 targeted therapy. sEphB4 may thus be particularly effective when combined with Her2 targeted therapy or even more likely to be effective if Her2 is mutated and non-responsive to the tyrosine kinase inhibitor. Thes approaches will need to be studied in human trials.

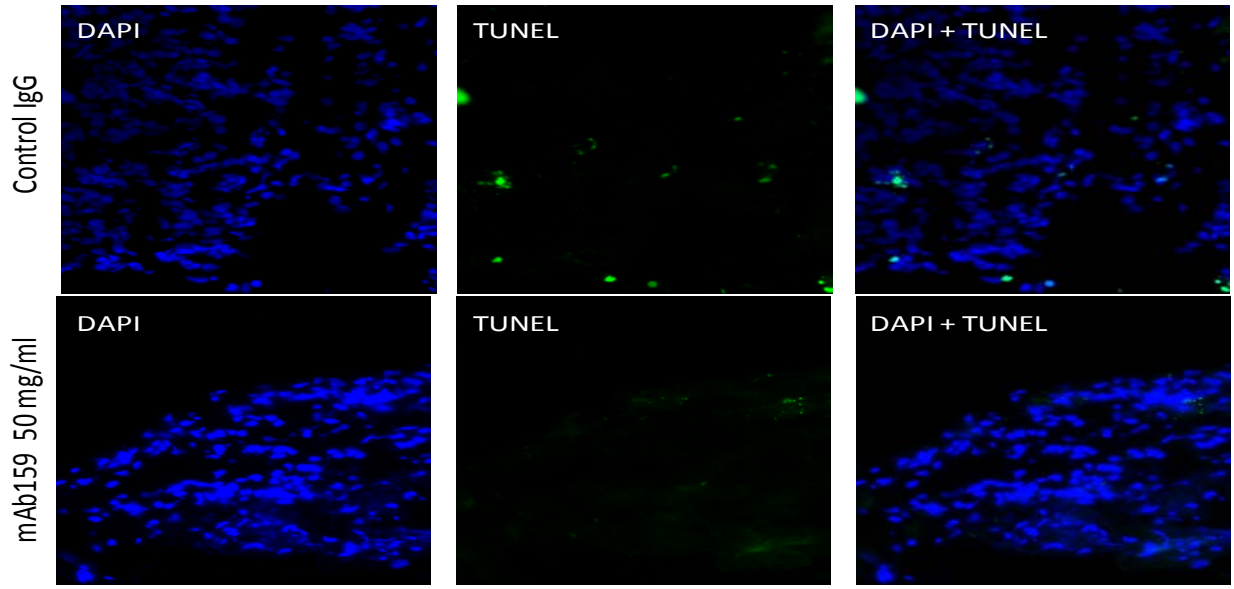


Task 3G: sEphB4 has direct antitumor activity in Her2 positive human tumor. In order to further document the potential utility of EphB4 targeted therapy in Her2 positive tumor, we studied freshly collected human tumor tissue and conducted ex-vivo efficacy study. sEphB4 or other test compounds were incubated with the tumor and 24 hr after treatment the tissue was harvested, processed and studied for apoptosis using TUNEL assay. Her2 positive tumor showed marked apoptosis after sEphB4 treatment, while there was no apoptosis in control treated tumor. These results demonstrate the direct tumor specific effect of sEphB4 in Her2 positive tumors.

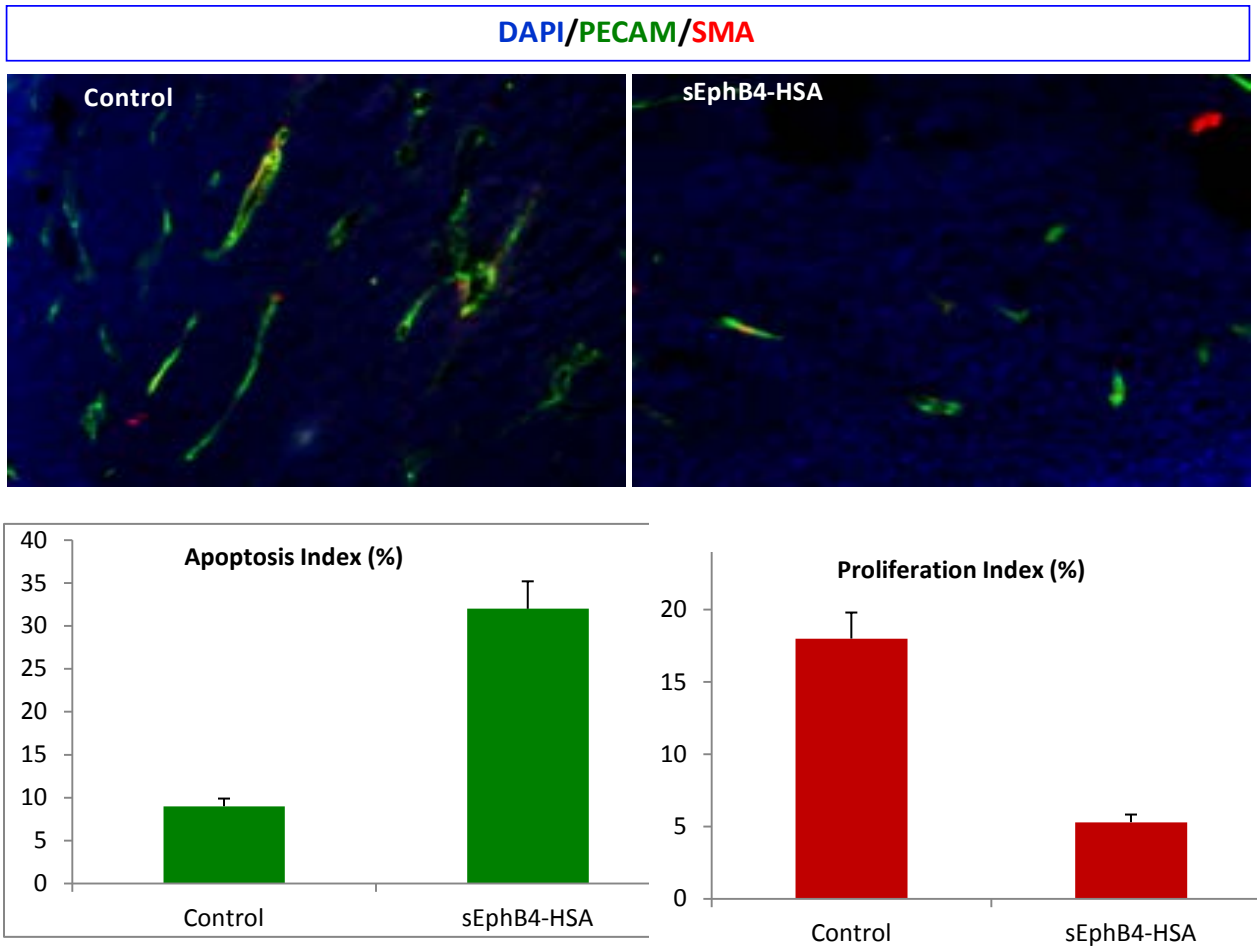
sEphB4-HSA induces apoptosis of human breast cancer 5789 tissue sections in 3-D histoculture



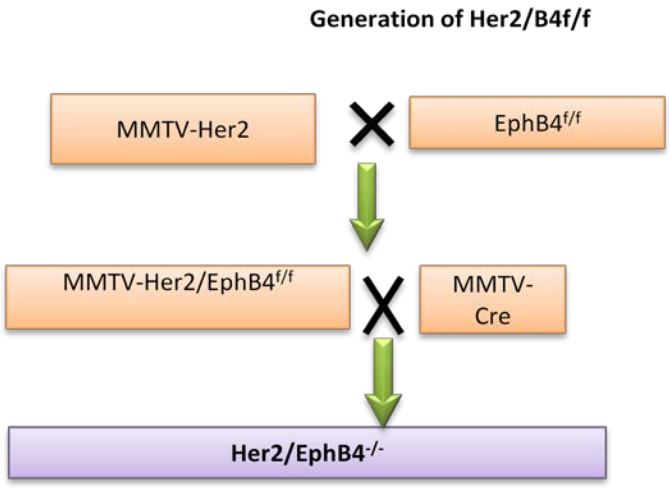
mAb159 does not induce apoptosis of human breast cancer 5789 tissue sections in 3-D histoculture



Task3H: Analysis of tumor and normal tissue from above studies for activated HER2/HER family receptors, angiogenic markers, vessel density, signal transduction (PI3K, Akt, mTOR, S6), proliferation (Ki-67) and apoptosis (TUNEL) Tumor samples from the control and treated tumor samples were studied for various markers. sEphb4 markedly inhibited PI3K pathway including pAkt, pS6, Ki-67 and induced cell death by apoptosis.



Task 4.A: Generate and study mammary tissue-specific EphrinB2 and EphB4 knock-out mice using MMTV-Cre system: The schema for the generation of Her2 transgenic mouse and concurrent deletion of EphB4 in the mammary gland is outlined below. Similar approach is applied to the development of EphrinB2 knock out in the mammary gland and crossed with Her2 overexpression to produce mammary tumor.



Task 4 B: Deletion of EphB4 and mammary gland: Deletion of Ephb4 in mammary gland did not alter the development of mammary gland.

Task 4 C. Determine the impact of EphB4 knock out on the time to tumor development, and rate of growth and risk of lung metastasis when mice from above task are crossed with NDL HER2 transgenic mice. Preliminary data reveals marked decrease in the development of tumor to less than 50% compared to 100% of controls, tumor lesions 36% of control. Survival is increased and a median has not yet been reached.

Genotype	**Survival (preliminary data)**	
	Number	Median survival (days)
MMTV-Her2;	12	175
MMTV-Her2;MMTV-Cre; EphB4--/-	19	236 +

Task 5. Formulation of next steps to bring co-targeting of EphB4 and HER2 in HER2+ breast cancer

Significant progress has been made toward the clinic. An IND has been obtained for sEphbB4-HSA, an albumin stabilized soluble EphB4 decoy receptor that efficiently blocks EphB4/EphB2 signaling. A phase I study for solid tumors using this agent given intravenously is now open and accruing patients, with one head and neck tumor response seen.

KEY RESEARCH ACCOMPLISHMENTS:

- Characterization of demographic, ethnic, clinical, treatment, genetic predisposition variables in patients with HER2+ breast cancer receiving neoadjuvant therapy, determination of response rate and clinical predictors of response to therapy (see reportable outcomes).
- Optimization of antibodies and conditions to stain for several antigens on very small amounts of core biopsy tissue
- Development of a neoadjuvant tissue repository, currently with 102 total cases and 42 HER2+ cases, and now to 72+ HER2+ cases identified. This resource is unique in that pre and post treatment tissue is available with highly annotated immunohistochemical data. Also, a database with the demographic, clinical, treatment and pathological information is linked to the tissue repository.
- Movement of EphB4 targeted soluble peptide (sEphB4-HSA) to the clinic with initiation of Phase I trial in solid tumors as a single agent.

REPORTABLE OUTCOMES:

1. Abstract presentation of the clinicopathological aspects of our neoadjuvant cohort that is supporting the tissue based studies :

   Tripathy D, Ahmed S, Bahl P, Wang Y, Ji L, Ricker C, Weng Grumley J, Liu SV, Sener SF, Klipfel N, Kaur C. Neoadjuvant therapy response, subtype and BRCA status in an underserved population. 34th Annual San Antonio Breast Cancer Symposium (Abstr P3-14-21), 2011.

2. We have successfully applied for a proposal that is related to this strategy to co-target EphB4 and the Notch receptor, using an in-house developed antibody to the tumor-specific Notch ligands DLL-1 and DLL-4. (California Breast Cancer Research Program Project entitled “Co-Targeting the Notch and EphB4 Receptor in Breast Cancer” (CBCRP 18IB-0048). This project received the highest scientific score of all review submissions. Work on this project is now proceeding with cell line data being generated on the combination EphB4 and DLL-1, 4 targeting with and without chemotherapy and animal studies initiated.

CONCLUSION: Patients in our population (primarily Hispanic) exhibit the same patterns of response to neoadjuvant chemotherapy for breast cancer, with higher pathological compete response (pCR) rates seen in triple negative and HER2+ breast cancer, and with higher pCR rates with the use of trastuzumab when added to chemotherapy for HER2+ breast cancer. In

the trastuzumab treated cohort, there were no biomarkers that predicted pathological response, but VEGFR1 did change significantly from pre to post treatment in the nonresponders, as did (as expected) Ki67. In the overall cohort treated with chemotherapy with or without trastuzumab, again no biomarker predicted response, but VEGFR1 and PDDGR as well as Ki67 decreased following therapy. This suggests that downstream markers of EphB4 are modulated downward by therapy, although it is not clear that this effect is clearly mediated via EphB4/EphB2. Together, these will lay the foundation for clinical trials co-targeting EphB4 and HER2. EphB4-targeted therapy is now here with a Phase I trial approved our institution with our in-house developed inhibitor, a ligand-blocking soluble albumin-stabilized EphB4 peptide termed sEphB4-HSA, so we are well positioned to transition this strategy of combined EphB4 and HER2 blockade, or co-targeting to the clinic.

REFERENCES:

1. Tripathy D, Ahmed S, Bahl P, Wang Y, Ji L, Ricker C, Weng Grumley J, Liu SV, Sener SF, Klipfel N, Kaur C. Neoadjuvant therapy response, subtype and BRCA status in an underserved population. 34th Annual San Antonio Breast Cancer Symposium (Abstr P3-14-21), 2011.

APPENDICES:

Appendix 1: Poster of above referenced abstract.